Patients and Methods: Nineteen pts with a median age of 56 years (range 18–70) not eligible for ablative SCT received Campath-1H 20 mg/m^2, fludarabine 25 mg/m^2 × 5 days and either cyclophosphamide 1 g/m^2 × 2 days (n=16 pts), melphalan 140 mg/m^2 (n=1 pt) or busulfan 0.8 mg/m^2 × 8 doses (n=2 pts) followed by G-CSF stimulated peripheral blood (n=13) or unmanipulated bone marrow(n=6). GVHD prophylaxis consisted of CSA at least until T+60 and mycophenolate mofetil through T+30. DLI was allowed for residual/progressive disease or mixed chimerism after T+60 in absence of GVHD. Seven pts had a previous autologous transplantation. Ten pts had myeloid diseases (AML=6, CML Ph negative=1, CML accelerated phase=1, therapy related MDS=1, myelofibrosis=1); 9 pts lymphoid (HD=3, NHD=5, PLL=1); only 5 pts were in CR at transplant. Six pts received bone marrow with a median CD34$^+$ cells infused of 3.7 × 10^6/kg, and 13 pts peripheral blood with a median of 4.5 × 10^6/kg CD34$^+$ cells infused. **Results:**19 achieved ANC >0.5 × 10^6/L within a median of 12 days (range 8–16); 2 pts rejected the graft and 1 had autologous reconstitution; 10/19 achieved sustained platelet engraftment at a median of 16 days (range 7–27). 2/17 pts with sustained engraftment developed acute GVHD; 7/14 at risk pts had CMV reactivation; TRM at T+100 was 16% (disease progression in 2 and GVHD in 1). Nine pts received DLI (5 for persistent mixed chimerism and 4 pts for persistent/progressive disease). After DLI 5 pts had GVHD limited to skin. Of the pts with myeloid malignancies 2 pts remain alive in CR at T+28, +19 months; of the pts with lymphoid malignancies 2 pts with HD are alive in CR at T+34, +15 months and 2 pts with mantle cell lymphoma are alive at T+19, T+13 months. Causes of death include disease progression in 7 pts, CMV disease in 1 pt, adenovirus/CMV disease in 1 pt, sepsis in 1 pt and GVHD in 3 pts. **Conclusion:** These results suggest that this approach is well tolerated with a low early TRM even in pts with advanced disease. Longer follow-up and better patient selection are needed.

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UMBILICAL CORD BLOOD TRANSPLANTS (UCBT). EVALUATION OF CHIMERISM AND SURVIVAL IN RELATION TO CD34 OR MONONUCLEAR CELL (MNC) DOSE INFUSED. THE CHILDREN'S MEMORIAL HOSPITAL (CMH) EXPERIENCE

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To evaluate chimerism and to determine if cell dose/kg has a relationship with time to full chimerism in children undergoing Hematopoietic UCBT unrelated (n=85) related (n=3) at CMH; between 1995 and 2004, one hundred and one UCBT were performed at CMH of those 88 meet the study criteria (survive >30 days post transplant) to be evaluable for engraftment and survival. There were patients (pts) with malignant (n=71) and non-malignant (17) diseases with a mean age of 5.0 years (.6–16) and a mean weight of 20.2 kg (5.6–61). The preparative regimen consisted of fTBI (1200 cGy) day −8 to −5, VP-16 1000 mg/m^2 day −4 and cyclophosphamide 60 mg/kg/day days −3 to −1. GVHD prophylaxis consisted of CSA, short course MTX, ATG (days 1, 3, 5, 7). Engraftment was defined as the time to reach >95% donor chimerism, assessed by either fluorescence in-situ hybridization or variable number tandem repeats (VNTRs). Cell counts were measured by the Abbott Cell Dyne counter and the CD34$^+$ cells were quantified by flow cytometry on a FACS sorter. Pts were divided in two groups according to cell doses received (<.7 or >.7) for CD34$^+$ × 10^6/kg and MNC × 10^8/kg cells. Statistical analysis was made by a non-parametric t test and column statistics (mean ± SEM and all were at the 95% CI) on (Graph Pad). The overall engraftment of the 88 patients was 72% (63/88). There was no difference among the groups in volume infused, malignant or non-malignant or the status of disease prior to transplant. Pts with the lower CD34$^+$ and MNC cells infused were heavier and showed a lower incidence of full chimerism and a trend to slower engraftment. There was also a survival difference in favor of the higher CD34$^+$ cell infused group.

Table 1.	CD34$^+$ × 10^6/kg			MNC × 10^8/kg		
Cell Doses	<.7	>.7	p Value	<.7	>.7	p Value
Age	6.8 ± 0.8	4.0 ± 3.6	0.002	6.6 ± 0.5	2.2 ± .45	<0.0001
Weight	25.7 ± 2.4	17.2 ± 1.3	0.0015	24.8 ± 1.6	12.5 ± 1.1	<0.0001
Days to >95% chimerism	37.3 ± 4.6	30.8 ± 2.9	0.2	34.8 ± 3.3	29.6 ± 3.7	0.3
% achieving full chimerism	52.9	83.3	0.001	56.8	78.0	0.02
% Overall survival	44.1	61.6	0.001	53.4	56.6	n/s
Number of patients	34	54		58	30	

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HEMATOPOIETIC CELL TRANSPLANTATION (HCT)-SPECIFIC-COMORBIDITY INDEX: A NEW TOOL FOR RISK ASSESSMENT BEFORE ALLOGENEIC HCT

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We have reported on the use of Charlson comorbidity index (CCI) to predict non-relapse mortality (NRM) and overall survival (OS) for patients (pts) given nonablative or ablative HCT[1]. However, the sample size of pts with scores of ≥1, captured by the CCI, did not exceed 35%. Further, some of comorbidities were rarely found among pts given HCT. Therefore, we sought to develop an HCT-specific-comorbidity index aimed at a) better defining previously identified comorbidities, i.e. adding pulmonary functions tests to pulmonary, liver function tests to hepatic, and ejection fraction ≤50% to cardiac comorbidities and b) investigating additional HCT-related comorbidities. To this end, we retrospectively reviewed comorbidities of 1055 pts given HCT at our center between 1997–2003 after nonablative (n=294) or ablative (n=761) conditioning. Pts were randomly divided into training (n=708) and validation sets (n=347). In the training set, the unadjusted hazard ratios (HR) for 2-year NRM were calculated for each comorbidity and then adjusted for other comorbidities, disease risk, and conditioning type. The adjusted HRs were employed as weights for individual comorbidities. Differences encountered compared to the original CCI were: a) two new comorbidities were added (obesity = score 1 and peritransplantation infection = score 2), b) age ≥50 years acquired a score of 2, and c) hypertension and asthma each acquired a score of 1 instead of 0, moderate pulmonary, peptic ulcer, and rheumatologic each acquired a score of 2 instead of 1, valvular heart disease a score of 2 instead of 0, and severe pulmonary comorbidity a score of 3 instead of 1. In the training set, HR for NRM for scores 0, 1, 2, 3, 4, ≥5 were 1, 1.2, 3.5, 6.1, 7.1, and 10.8, respectively. The modified index was then validated using the validation set. This index had the advantage of 1) capturing more pts with high scores and 2) distinguishing pts with low scores who had lower NRM and better OS when compared to the original CCI (Table). Applying the scores to nonablative and ablative pts, respectively, NRM of 5 vs 10% (p=0.4) and OS of 85% vs 75% (p=0.1) were seen for scores of 0–1, 17 vs 27% (p=0.04) and 61 vs 59% (p=0.2) for scores of 2–3, and 33 vs 54% (p=0.03) and 43 vs 30% (p=0.006) for scores of ≥4. This HCT-specific comorbidity index provides a simple, readily applicable and valid method of estimating NRM and OS among pts given nonablative or ablative allogeneic HCT. (1. Sorror et al. *Blood.* 2004; 104:961.)

Table. *Percentages of pts and Two-Year Rates of NRM and OS in the Validation Set (n = 346) as Scored by the New HCT-Specific Comorbidity Index Compared to the Original CCI*

		Two-Year Rate				Two-Year Rate	
HCT-CI	pts, %	NRM, %	OS, %	Original CCI	pts, %	NRM, %	OS, %
0–1	37	13	72	0	58	17	64
2–3	36	22	58	1	27	33	51
≥4	27	40	35	≥2	15	31	41

NRM indicates non-relapse mortality; OS, overall survival; CCI, Charlson comorbidity index; and HCT-CI, hematopoietic cell transplantation-specific comorbidity index.

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EFFECTS OF NATURAL KILLER (NK) CELLS ON ALLOGENEIC BONE MARROW TRANSPLANTATION (BMT) IN FETAL AND NEWBORN MICE

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NK cells are capable of receptor-mediated lysis of target cells that lack self class I MHC molecules. Thus, it is possible that they can be used effectively in an MHC I mismatched allogeneic BMT to create myeloablation without developing GVHD. We wanted to define the role of the NK cell as a myeloablative agent. C57Bl/6 (B6) adult NK cells were injected i.p. into 2 day old Balb/c newborns. At various times post transplant, bone marrow was harvested and in-vitro colony forming assays were done. We found that allogeneic NK cells destroyed both erythroid and myeloid progenitor cells by 5 days post injection with some recovery by 19 days. We then injected NK cells with/without bone marrow from C57Bl/6 (B6) mice into 14 day old Balb/c fetuses and newborn animals. We found that none of the fetal recipients survived the *in utero* transplants even when the number of NK cells was reduced to 10,000 cells. Histologic studies suggested severe liver toxicity associated with the fetal injections. In contrast, 7/10 newborn recipients of allogeneic lin^- BM and NK cells had multi-lineage engraftment 8 weeks post transplant (Table). The engraftment was inhibited by co-administration of anti NK1.1 mAb or anti-TGF beta antibody suggesting that NK cells play a role in the engraftment and mediate their effect at least partially through the secretion of TGF-beta. We used an Artemis deficient $T^-B^-NK^+$ mouse to study the repopulation of the marrow following transplant with NK cells. We found significant repopulation of T cells (36 ± 17% vs 7 ± 2% control) in blood and thymus with evidence of B cell reconstitution in bone marrow. NK cells can be used effectively as a means of enhancing donor cell engraftment and in some circumstances inducing multilineage engraftment. This work was supported by a grant from the NIH NIAID RO1 HL58842 (Table 1).

Engraftment (Percent) in Newborn Balb/c Recipients of B6 NK Cells and Lin^- Bone Marrow at 8 Weeks

Groups	Animals Engrafted	$CD3^+$ Cells	Granulocytes	B Cells	Monocytes	NK Cells
BM Alone	0/4	0.39 ± 0.11	0.1 ± 0.02	0.1 ± 0.04	0.1 ± 0.02	None detected
NK + BM	7/10	16.5 ± 6.3	0.5 ± 0.7	2.6 ± 1.0	1.4 ± 1.8	2.1 ± 0.6
NK Alone	3/10	None detected	None detected	None detected	None detected	5.11 ± 1.4

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LONG TERM INFECTIOUS COMPLICATIONS FOLLOWING REDUCED INTENSITY CONDITIONING (RIC) ALLOGENEIC HLA-IDENTICAL SIBLING TRANSPLANTATION (ALLO-SCT)

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We analyzed infections occurring beyond 6 months after allo-SCT, in 81 consecutive pts who received a RIC prior to allo-SCT from HLA-identical siblings: median age was 49 (18–63) years. 33 (41%), 34 (42%) and 14 (17%) pts had respectively a myeloid, lymphoid or non-hematological malignancy. 62 pts (77%) had an advanced disease with high risk clinical features precluding the use of standard myeloablative allo-SCT. 21 pts (26%) BMT and 60 (74%) PBSCT. RIC consisted of fludarabine and busulfan with ATG (high dose: 20 (25%); low dose: 50 (62%)) or of low dose irradiation-based RIC (N=11, 13%). With 26 (9–68) months follow-up, 67 pts (83%) experienced at least one infectious episode beyond the first six months after allo-SCT developing at a median of 8 (6–34) months. 221 infectious episodes were observed, of which 123 (56%) required hospitalization and systemic anti-microbial therapy. 94 episodes (43%) could be documented (bacterial, n=28; viral, n=56; fungal, n=10). Documented infections were: gram negative bacteria (16%), other bacteria (14%), CMV positive antigenemia (17%), HSV (25%), VZV (15%), other viruses (3%), aspergillus (4%), candida species (6%). 81% pts (n=54) with an infection were under systemic immunosuppressive therapy for chronic GVHD. Moreover, 24 pts (30%) experienced >1 episode of documented infections (median, 2 episodes; range, 1–12). In a univariate analysis performed in the pts with documented infection, age <50 (P=0.06), the use of PBSCs as stem cell source (P=0.03) and a history of DLI (P=0.002), exerted a protective effect against infection occurrence. In a multivariate analysis, DLI was significantly associated with a decreased risk of long term infections (P=0.002; RR=0.23; 95% CI, 0.1–0.6), indirectly highlighting the protective effect of donor origin immunity after allo-SCT. In this series of pts surviving at least 6 months after RIC allo-SCT, the overall long term transplant-related mortality was 14% (n=11), of whom 6 deaths were attributed to infections (3 bacterial septicemia, 2 aspergillosis, 1 pneumocystosis), giving a cumulative incidence of long term-infectious related mortality of 7% (95% CI, 1.4–12.6%). Obviously, prospective efforts to develop optimal antimicrobial preventive strategies after RIC allo-SCT are still needed. However, the results from this analysis compare favorably with data from the standard myeloablative allo-SCT setting.

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DRAMATIC CHANGE IN TREATMENT RELATED MORTALITY (TRM) FOR ALLOGENEIC STEM CELL TRANSPLANTATION (SCT) DURING RECENT YEARS. A ONE CENTRE ANALYSIS

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Background: During recent years it has been the impression in many centres that TRM following allogeneic stem cell transplants has declined significantly. This impression has been strong in our centre as well. The aim of the present study was to analyze the magnitude of this presumed reduction in procedure related risks and to examine possible causes. **Method:** We analyzed a recent time period of ten years from Jan. 1994 to Jan. 2003. Non myeloablative transplants were excluded from the study. During this period 224 and 219 standard allotransplants were performed during the first and the second 5-year time periods respectively. All transplants were performed at the same centre (Copenhagen, Denmark), whose organization has essentially remained the same for the whole time period. **Results:** TRM differed significantly (p<0.0001) between the two time periods. The one-year Kaplan-Meier based TRM declined from 33.5% to 13.9%. On the basis of univariate analysis no significant differences was found between the compositions of the main diagnosis (AML, ALL, MDS, CML, non-malignant). Likewise, when the pre-transplant stages of acute leukaemia and CML were compared between the two periods non differences were recorded, except that slightly less very high risk ALL patients were transplanted during the last period. No difference in age distribution was found. In contrast, a tendency for a more restrictive donor selection policy could be recorded. Hence, the composition of donor types (HLA-id Sib vs. other fam. donors vs. UD; p=0.007), donor gender (less female to men; p=0.018) and the use of class I high-resolution donor typing for UD (p=0.0001)